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TITLE: Topical Modulation of the Burn Wound Inflammatory Response to Improve Short and Long Term Outcomes

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13. SUPPLEMENTARY NOTES**14. ABSTRACT**

We propose to investigate the relationship between p38MAPK signaling, wound inflammatory response, wound healing and long-term scar formation using a burn model in the female red Duroc pig. We hypothesize that topical p38MAPK inhibition will attenuate the depth of the burn by preventing hair-follicle cell apoptosis, attenuate the inflammatory phase of wound healing, and decrease the granulation layer thickness. We propose this modification in the early inflammatory response will also reduce thickness and contraction of scars formed after deep partial thickness burn injury. The knowledge gained from our proposed research will be critical to implement a potential paradigm shift in the clinical treatment of challenging dermal injuries.

15. SUBJECT TERMS

None provided

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Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	5
4. Impact.....	10
5. Changes/Problems.....	10
6. Products.....	11
7. Participants & Other Collaborating Organizations.....	11
8. Special Reporting Requirements.....	12
9. Appendices.....	13

1. INTRODUCTION:

Approximately 500,000 Americans suffer burn injuries with an estimated 3,500 deaths annually. Widespread makeshift bombs contribute to burns and large wounds being one of the significant causes of warfighter casualties. The magnitude and impact of burns can be devastating as large numbers casualties occur simultaneously. Secondary organ damage and failure frequently occurs after injury. Moreover, wound complications such as hypertrophic scars may cause significant morbidity, disabling loss of function, extended difficult recovery times, dramatically affecting the patient's quality of life physically. We are investigating a topical therapy that is easy to apply and can be used by a wider range of health care providers in a mass-casualty incident.

2. KEYWORDS:

Wounds, Burn, topical, wound healing, inflammatory signaling, Mitogen activated protein kinase, hypertrophic scar, p38, combat casualty, treatment, organ failure, systemic inflammatory response syndrome, thermal injury, wound model, intervention

3. ACCOMPLISHMENTS:

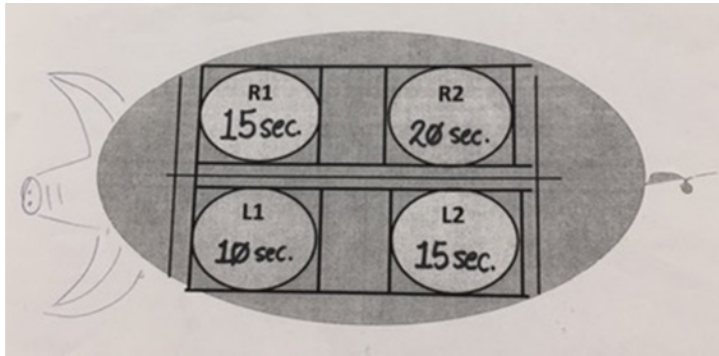
What were the major goals of the project?

1. Establish the female red Duroc pig model burn model as the appropriate wound healing model that resembles human response. At the end of the project, we will have a well-defined animal model for human wound healing. This animal model may provide a tool that other investigators can use to screen for compounds that may modify wound healing and reduce scar formation. The ability to have a standard animal model for wound healing may bring an exciting new era in the investigation and elucidating the molecular mechanisms of hypertrophic scar pathophysiology and developing therapeutic agents.
2. Define the inflammatory signaling post burn injury and elucidate the relationship between early inflammatory signaling, wound healing, and scar formation.
3. Define the role of p38MAPK in wound healing and scar formation.
4. Identify the wound healing response to topical p38MAPK inhibition. Demonstrate early wound healing and reduced scar formation with topical p38MAPK inhibition. Define the long-term wound outcome of topical p38MAPK inhibition post-burn injury.
5. Identify the optimal timing and duration of treatment for p38MAPK therapy.
6. By the end of the project, have a well-defined protocol and experimental plan to initiate human subject research to study topical p38MAPK inhibition as a therapy to decrease end-organ dysfunction, improve wound healing, and reduce scar formation in patients with burn injuries.

What was accomplished under these goals?

Goal 1. Establish the female red Duroc pig model as the appropriate wound healing model.

The first goal of the project was to establish the female red Duroc pig model burn model as the appropriate wound healing model that resembles human response. Our first 3 porcine experiments, porcine group (Pg) 001-003, were the dermatome model, demonstrating that the female red Duroc porcine model significantly correlates to human hypertrophic scarring. We started our experiments using the burn wound model with Pg004. In this model we use a 'hot water bottle' thermal injury device. Briefly, we use a 500 ml Pyrex laboratory Schott Duran bottle with the bottom glass removed, edges smoothed, bottom replaced with cling wrap, and secured with heat resistant tape. The bottles will be filled with 300 ml of water and then heated to the desired temperature of 92°C. We have improved our technique significantly over the period of the current grant. The initial wounds were not uniform. Starting with Pg 007 (Table 1), we have resolved the technical issues and burn wounds are uniform (Figure 1 is from Pg011 experiment, the last animal experiment).



We change the depth of the burn by changing the length of the contact for 10, 15, and 20 seconds. We have identified that all these contact times are in the range of partial thickness injury. The 20 seconds is mostly very deep partial thickness to full thickness burns (please see the Optical Coherence Tomography data in goal 2). The 15 seconds burn is our focus with partial thickness burn injury. Goal 1 is accomplished during the year 1 period September 2014-September 2015.

Goal 2: Define Inflammatory Signaling Post Burn Injury and Elucidate Relationship...

The second goal of the grant was to define the inflammatory signaling post burn injury and elucidate the relationship between early inflammatory signaling, wound healing, and scar formation. Rather than systemic modulation of the inflammatory response, we propose a novel approach, which calls for "inflammatory source control". We define "inflammatory source control" to be all the maneuvers that can be used to control a focus of inflammation, which is thought to be the initial source of systemic immune activation. In our burn model, we control the source of inflammation by application of a topical p38 MAPK inhibitor. The p38 MAPK pathway is the key inflammatory intracellular signaling in mammalian cells. In our previous murine models, we demonstrated that topical application of p38MAPK inhibitors after burn injury attenuated wound inflammatory response and stress signaling, leading to reduced systemic

inflammatory activation and end-organ dysfunction (these data already published and not part of the current investigation). In our porcine model, our goal is to demonstrate that topical p38 MAPK inhibitors will attenuate wound inflammation and reduce scarring in the red Duroc pig model of fibroproliferative scarring. We have done total of 11 pig experiments. The porcine group experiments are numbered 001-011 (Table 1).

We have analyzed these wounds using several different methods:

- Wound character: time to wound closure, color, wound infection
- Histopathology: H&E, TUNNEL assay,
- Inflammatory and wound healing gene expression
- Custom porcine RT-qPCR Array
- Optical Coherence tomography (OCT)

Table 2 demonstrates all completed, in-progress, and pending analyses (N/A is not applicable; meaning that set of analysis will not be done; for instance, no wound closure analysis will be done in 3 day experiments).

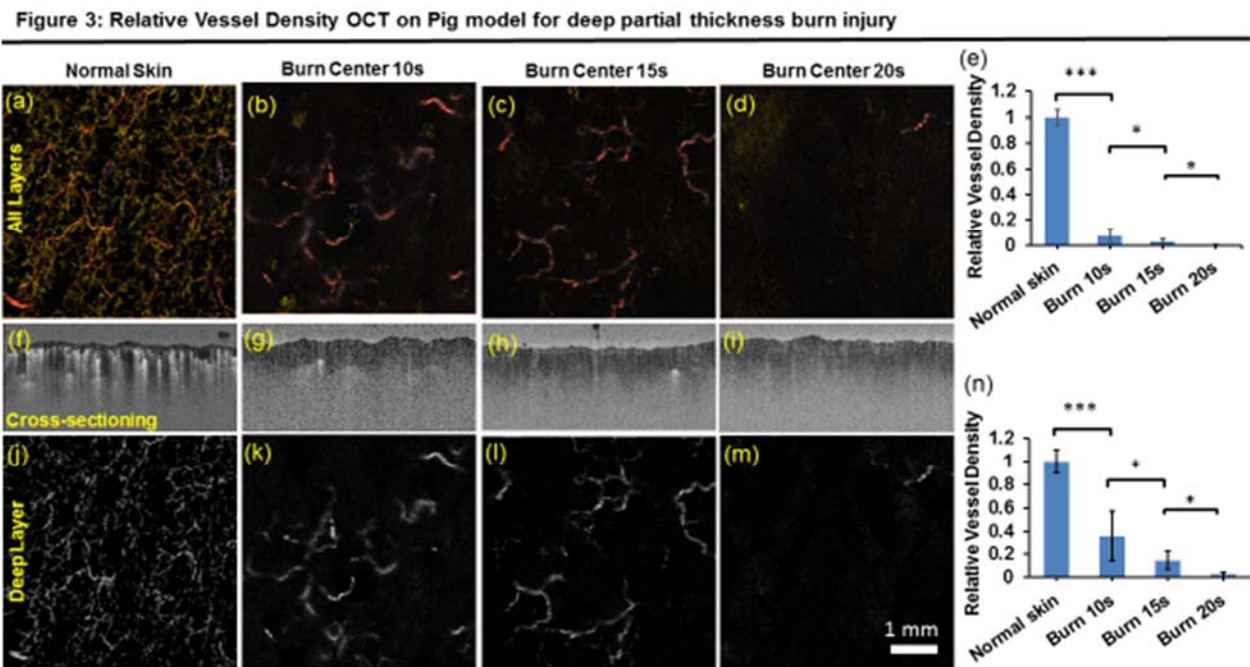
We have used porcine RT-qPCR Array for wound healing, inflammatory, and apoptosis pathways. We have demonstrated a difference in pattern of pathway expression between the burn versus non-burn skin and burn treated with p38 inhibitor versus vehicle. In the burn wound healing, there is a portion of collagen arrangement that remains intact. In the dermatome model the line between “normal” collagen and “abnormal” collagen is sharp and clear. In the burn model there is a large transition zone between the intact dermal architecture and damaged skin. This reflects the ongoing inflammation and apoptosis seen in burn injury. When we examined the gene expression differences in various depth of injury, an interesting pattern was observed. The deeper burns were associated with increasing number of over-expression of the regulatory genes. This internal consistency in the model is very important (already shown in the second year report).

Table 1 Study ID	Wound	Dates	Treatment	Pigs #	Duration of study
Pg001	Dermatome	Feb-10	Topical p38MAPK inhibitor versus control	3	20 weeks
Pg002	Dermatome	Sep-11	Topical p38MAPK inhibitor versus control	4	20 weeks
Pg003	Dermatome	Sept 2012-Oct 2012	PGE2 agonist topical versus control	12	3 weeks
Pg004	Scald Bottle Burn	Sept 2013- Oct 2013	Topical p38MAPK inhibitor versus control	6	2 weeks
Pg005	Scald Bottle Burn	May 2014	Topical p38MAPK inhibitor versus control	6	2 weeks
DoD Grant Funded following:					
Pg006	Scald Bottle Burn	Dec 2014	Topical p38MAPK inhibitor versus control	6	3 days
Pg007	Scald Bottle Burn	May 2015	Topical p38MAPK inhibitor versus control	6	3 days
Pg008	Scald Bottle Burn	Sept-Oct 2015	Topical p38MAPK inhibitor versus control	8	2 weeks
Pg009	Scald Bottle Burn	May 2016	Topical p38MAPK inhibitor versus control	8	3 days
Pg010	Scald Bottle Burn	May 2017	Topical p38MAPK inhibitor versus control	8	2 weeks
Pg011	Scald Bottle Burn	Oct 2017-Feb 2018	Topical p38MAPK inhibitor versus control	6	20 weeks

Table 2	Assay Status							
Study ID	H & E Slides	Wound Closure Images	Itch Score	Wound Contracti on Images	Wound Healing Profiler RT2-qPCR Array	Apoptosis Profiler RT2-qPCR Array	Cleaved Caspase3 (CC3) Immuno Histo chemistry	Western Blot: p38 MAPK activatio n, and CC3
Pg001	Completed- Analysis	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Pg002	Completed- Analysis	n/a	n/a	Completed - Analysis	n/a	n/a	n/a	n/a
Pg003	Completed- Analysis	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Pg004	Completed- Analysis	Completed- Analysis	n/a	n/a	Completed- Analysis	n/a	n/a	n/a
Pg005	Completed- Analysis	Completed- Analysis	subjective inconclusive	n/a	Completed- Analysis	n/a	n/a	n/a
Pg006	Completed- bx tissue processed thru H&E digital images. On hold- image measurements. Pending- western p38 activation results.	n/a	n/a	n/a	Completed- bx tissue biopulverized. On hold- pending western results	Completed- bx tissue biopulverized. On hold- pending western results	Completed- bx tissue processed thru slides On hold-pending western CC3 results	In progress
Pg007	Completed- bx tissue processed thru H&E digital images. On hold- image measurements. Pending- western p38 activation results.	n/a	n/a	n/a	Completed- Arrays. Completed- partial analysis (pending Pg009 arrays completion)	Completed- Arrays. Completed- partial analysis (pending Pg009 arrays completion)	Completed-bx tissue processed thru CC3 IHC digital images. Completed-RO1 analysis Completed- algorithm consult/design NWBS for R01 depth analysis On hold pending- western CC3 results	In progress
Pg008	Completed bx tissue processed thru H&E digital images. In progress- image measurements.	Complete	n/a	n/a	Completed- Arrays. Pending- final analysis formatting	n/a	n/a	n/a
Pg009	Completed bx tissue processed thru H&E digital images. On hold- image measurements. Pending western p38 activation results	n/a	n/a	n/a	Completed-bx tissue biopulverized In progress- arrays	Completed-bx tissue biopulverized In progress- arrays	Completed- bx tissue processed thru slides On hold-pending western CC3 results	In progress
Pg010	Completed bx tissue processed thru H&E digital images. In progress- image measurements.	In Progress	n/a	n/a	In prgoress- Arrays. Pending- final analysis formatting	n/a	n/a	n/a
Pg011	Completed bx tissue processed thru H&E digital images. In progress- image measurements.	Experiment in progress						

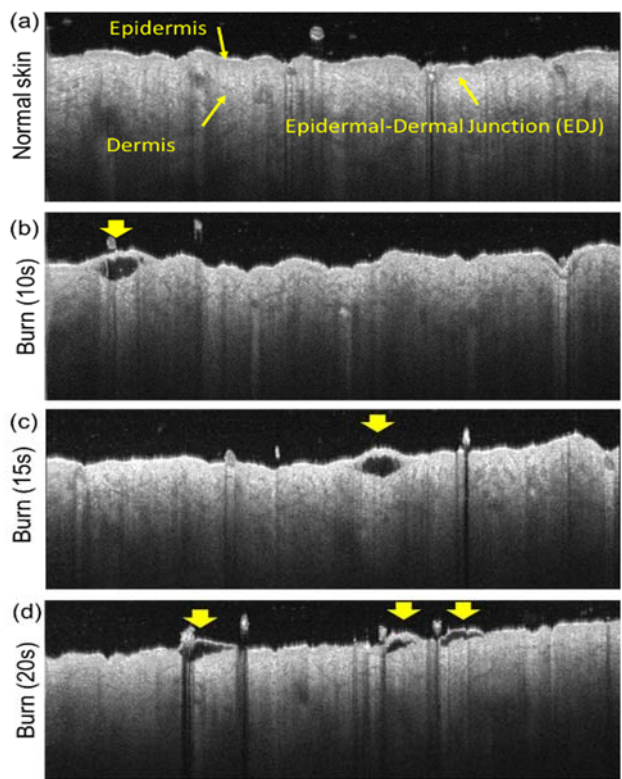
Optical coherence tomography (OCT) is a noninvasive diagnostic method that offers a view into the superficial layers of the skin in vivo in real-time. An infrared broadband light source allows the investigation of skin architecture and changes up to a depth of 2-3 mm with a resolution between 15 and 3 μ m. OCT provides a quick, non-invasive and useful diagnostic imaging technique for a number of clinical questions and is a valuable addition or complement to other analyses, such as H&E. We performed OCT analyses in our model after burns. The OCT demonstrated that the relative vessel density decreased with increasing depth of burn from 10

seconds to 20 seconds (Figure 3). The 20 second burn has almost no vessels, suggesting deeper burns.



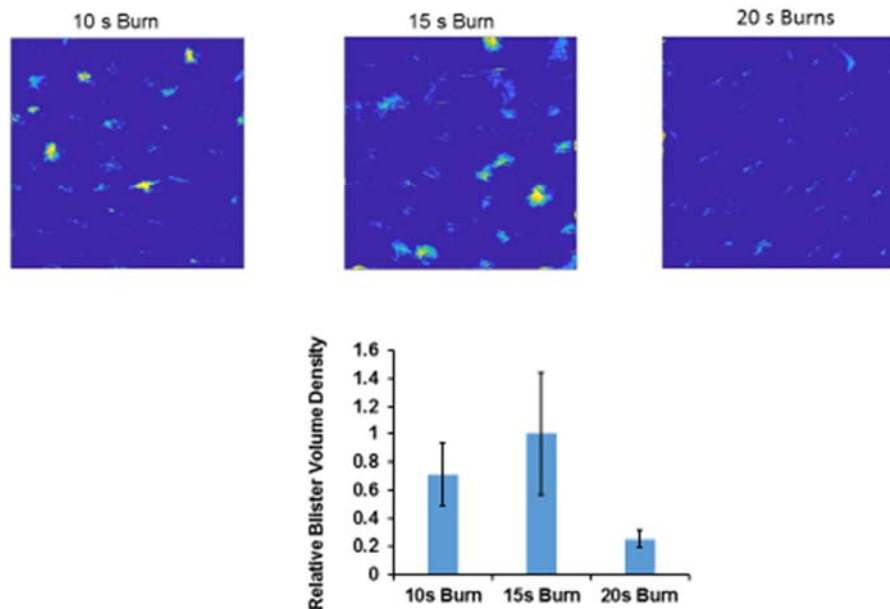
We also had an unexpected finding. In human burn injury almost all partial thickness burns have a blister response. Using human eye or H&E studies, we did not observe any blisters in the pig model. However, the OCT demonstrated that the porcine model has blistering response, as well (Figure 4, the arrow marks the blisters).

Figure 4



In human subjects, the deeper the partial thickness burn, the bigger the blister, until it gets close to full-thickness burns. The full-thickness burns are dry and without blisters, since the dermis is dead and leathery. In the porcine model, the OCT demonstrated that the 15 second burn has the maximum amount of blisters, confirming that the 15 second wound is the deep partial thickness wound (Figure 5). The 20 second model either has the most blisters (periphery) or no blisters. This suggests that the 20 second burn is occasionally full-thickness (in the center). In our model, we created one 10 s, one 20 s and two 15 s, since the goal was to focus on deep partial thickness

Figure 5: OCT Blister Volume Density Quantification



burns. Having blisters in the partial thickness wounds in the porcine model, demonstrates another similarity with the human subjects.

Goal 3: Define the role of p38MAPK in wound healing and scar formation.

Referring to table 2, a significant portion of goal 2 (Define the inflammatory signaling post burn injury and elucidate the relationship between early inflammatory signaling, wound healing, and scar formation) and goal 3 (Define the role of p38MAPK in wound healing and scar formation) has been done. However, final analyses and completion of work is pending for Pg010 and Pg 011. In the Quad chart, I marked as complete, since other than scarring, the rest is done.

Goal 4: Identify the wound healing response to topical p38MAPK inhibition. Demonstrate early wound healing and reduced scar formation with topical p38MAPK inhibition. Define the long-term wound outcome of topical p38MAPK inhibition post-burn injury.

We have demonstrated early wound closure with p38 MAPK inhibition. Using the dermatome model in Pg001 and Pg002, we demonstrated that wounds treated with p38MAPK inhibitor epithelialized faster than control group in 2 and 3 week time-points (Year 2 report).

The scar formation portion of this goal is pending. Experiment Pg011, our most recent experiment that is in progress, is the long-term outcomes portion of the project with direct analyses of the scar. The length of this experiment is 20 weeks, and the burn wounds are created

based on figure 1. In Pg011, we have 3 pigs in the control arm and 3 pigs in the p38 MAPK inhibitor treatment arm. The treatment arm had p38 MAPK inhibitor twice a day for 72 hours post burn injury. Wound photographs were taken at day 0, 3, 7, and weekly thereafter. Each photo is taken with a ruler to set image analysis scaling, and quantification will be performed using computer software ImageJ (ver. 1.50p, NIH, Bethesda, MD). Using the pictures we will analyze wound contracture. In addition to H&E and OCT imaging, we will have three experienced burn providers that are blinded to the treatment groups examine the wounds in vivo at multiple stages of the healing. They will score the scars.

Goal 4, as it relates to scarring, will be completed after the Pg011 experiment is finished and data analyzed; therefore, the current status is in progress. However, in the Quad chart this marked complete, since other than the final scar.

Goal 5: Identify the optimal timing and duration of treatment for p38MAPK therapy

The duration of p38 MAPK treatment has been set at 72 hours. We have used topical p38 MAPK upto 3 days after injury. There has been no increase in wound infection or delayed wound closure. Therefore, we concluded that 72 hour time point remains optimal. It remains to be seen, if longer time is associated with better outcomes (Year 2 report). This goal is accomplished.

Goal 6: By the end of the project, have a well-defined protocol and experimental plan to initiate human subject research to study topical p38MAPK inhibition as a therapy to decrease end-organ dysfunction, improve wound healing, and reduce scar formation in patients with burn injuries.

This is the final goal of the project. We are hoping by the end of the project to develop a human subject's study protocol. This goal is pending.

What opportunities for training and professional development has the project provided?

The collaboration with NWBiospecimen (Univ. of WA) has improved the understanding of apoptotic response in burn injury with both groups.

How were the results disseminated to communities of interest?

We are currently writing 3 manuscripts: one will be defining our model, another will be comparing p38 MAPK to control, and the third one will be the OCT findings. We will also prepare a grant for human subjects.

What do you plan to do during the next reporting period to accomplish the goals?

- 1- Complete Pg011, which is our last animal experiment.
- 2- Complete OCT and H&E analyses.
- 3- Complete and analyze the blinded scar examinations.
- 4- Write 3 papers, and prepare a human subjects grant

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We are learning about expressions of genes after burns. More importantly, the reduction of normal function in cells is very interesting.

What was the impact on other disciplines?

The impact of understanding inflammatory response and dermal pathology may be important in treatment of dermatological pathology, such as psoriasis.

What was the impact on technology transfer? We are working with our postdoctoral bioengineering group that is developing better OCT scanner. The OCT can be used in evaluation of skin lesions, especially non-melanoma skin cancers and inflammatory diseases, quantification of skin changes, visualization of parasitic infestations, and examination of other indications such as the investigation of nails. OCT provides a quick and useful diagnostic imaging technique for a number of clinical questions and is a valuable addition or complement to other noninvasive imaging tools for evaluation of burn wounds.

What was the impact on society beyond science and technology?

I hope in the future, we can develop a cream for burn victims.

5. CHANGES/PROBLEMS:**Changes in approach and reasons for change.**

There are no changes in the plan.

Actual or anticipated problems or delays and actions or plans to resolve them.

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

I am proud to state that during the 11 porcine burn experiments (6 after DOD grant), there has been no death before euthanasia or major issues for total of 54 pigs (42 pigs after DOD grant). We now have appropriate number of experiments. We have started our long-term (Pg011), 20 weeks study which will end February 2018, and there are no more new animal studies planned. We performed appropriate reduction of animals used. There has been no animal demonstrating that their pain is not adequately treated. The veterinary team uses our laboratory as an example of an outstanding group. We only used the minimum number of animals necessary with the focus on animal welfare issues.

Significant changes in use or care of human subjects.

Not applicable

Significant changes in use of biohazards and/or select agent.

None

6. PRODUCTS:

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Saman Arbabi, MD, MPH
Project Role: PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: He will provide the overall supervision and direct the animal studies.
Change in effort: From 10% to 8% as of June 1, 2015

Name: Adelaide Warsen, MS
Project Role: Research Scientist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Direct performance of all the proposed animal research
Change in effort: None

Name: Carina Morningstar
Project Role: Lab Technician
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 4
Contribution to Project: Research Assistance
Change in effort: Added at 50% as of January 9, 2017

Name: Kristen Huden
Project Role: Lab Technician
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Contribution to Project: Research Assistance
Change in effort: Added at 50% as of March 6, 2017

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No

What other organizations were involved as partners?

The collaboration with NWBiospecimen (Univ. of WA), Histology Imaging Core (UW), and

Fred Hutchinson Cancer Research Center (FHCRC) Experimental Histopathology Shared Resource is going well. We have not had the results yet, and if there are issues, we expect to resolve them with assistance of Dr. Schmechel (Chief of Pathology at Harborview medical Center and the Director for NWBiospecimen histology core).

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable

QUAD CHARTS: Attached

9. APPENDICES: None

Topical modulation of the burn wound inflammatory response to improve short- and long term outcomes



2b. Accelerated wound healing
PI: Sam Arbabi, MD, MPH

Award #W81XWH-14-1-0401
Org: University of Washington

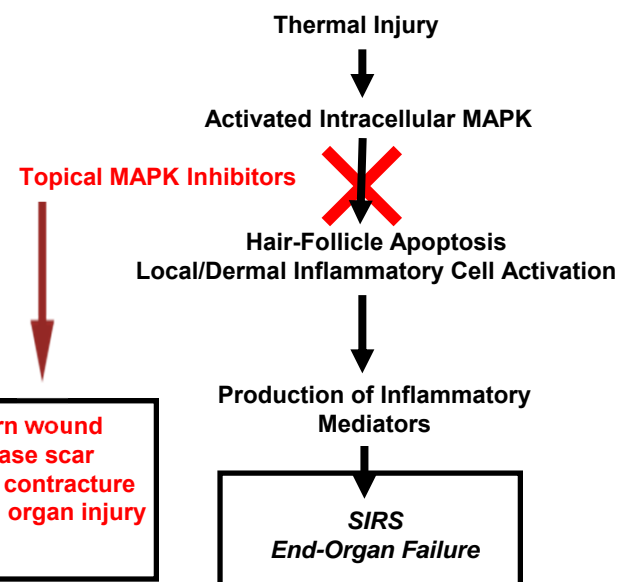
Award Amount: \$1,497,377

Study/Product Aim(s)

- The magnitude and impact of burns associated with warfare can be devastating. We employ a gel formulation of a powerful p38MAPK inhibitor that is ideal for early topical intervention on the battlefield, ready to use, and can be rapidly applied to wounds by self, buddy aid, and/or first responders. This topical treatment would aid in preservation and stabilization of systemic homeostasis, mitigating short and long term deleterious consequences of severe wound injuries, such as secondary organ damage, scar formation, and burn contracture.

Approach

- In the current application, we propose to continue our wound healing studies in an animal burn model that resembles human wound healing. We will use the red Duroc pig burn wound model that resembles human wound healing and scar formation.



Timeline and Cost

Activities	FY	14-15	15-16	16-17	17-18
Aim 1: Determine the effect of topical p38MAPK inhibition on wound healing gene expression in the female red Duroc pig model of burn injury					
Aim 2: Determine the effect of topical p38MAPK inhibition on early wound healing post-burn injury					
Aim 3: Define the long-term wound outcome of topical p38MAPK inhibition post-burn injury.					
Estimated Budget (\$K)		367	373	377	381

Updated: Oct 2017

Goals/Milestones

FY 14-15 Goals –

- ☒ Establish the red Duroc pig burn model

FY 15-16 Goals–

- ☒ Define the inflammatory signaling post burn injury
- ☒ Identify the optimal timing and duration of treatment for p38MAPK.

FY16-17Goals –

- ☒ Demonstrate early wound healing, decreased burn wound depth, and reduced inflammatory response with topical p38MAPK inhibition.

FY 17-18 Goal–

- ☐ Demonstrate decreased scar formation with topical p38 MAPK inhibition.

Comments/Challenges/Issues/Concerns

- If timelines change, comment here.

Budget Expenditure to Date

Projected Expenditure: \$190,000

Actual Expenditure: \$187,074